APPLICATION NOTE

Liquid Chromatography

AUTHOR

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HPLC Analysis of Acetaminophen, Aspirin and Caffeine Using LC 300 in Accordance with the USP

Introduction

Acetaminophen, aspirin and caffeine used in combination are an effective method of treating acute headaches and migraines. The combination of these substances is considered superior to acetaminophen on its own when treating headaches.[1] There are other benefits due to the individual components of the tablets. Acetaminophen (also known as paracetamol) is the most common analgesic and antipyretic around the world, it is an effective treatment for acute primary headaches on its own and can be combined with tramadol to treat cluster headaches. It has a very low risk of causing allergic reactions and so can be used by those with bronchial asthma.[2] Aspirin is a first-line therapy to treat moderate to severe primary headaches as well as reducing pain, fever and inflammation. However, it is not taken by children under twelve due to the risk of Reye syndrome. Caffeine is considered an effective treatment for various types of headaches including post-dural puncture headaches and hypnic headaches. It also provides an increase in high energy exercise tolerance and reduces fatigue. These drugs combined are commonly sold under the brand names Anadin Extra in the UK and Excedrin in the U.S. In 2019 approximately 13.1 million units of Excedrin Migraine were sold in the United States.[3]

This application brief describes the use of a LC 300 HPLC system with a UV/Vis detector for the analysis of acetaminophen, aspirin and caffeine (Figure 1) in accordance with the official Acetaminophen, Aspirin and Caffeine Tablets United States Pharmacopeia (USP) method for the assay.[4]

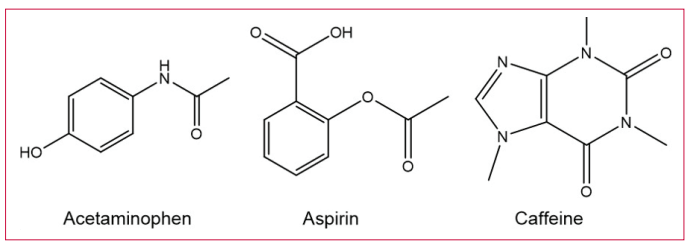

Figure 1. Structure of Acetaminophen, Aspirin and Caffeine.

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Experimental

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters.

Instrument	PerkinElmer LC 300 HPLC system with LC 300 multi-wavelength UV/Vis (MWD) Detector
Column	C18 100 x 4.6 mm, 5 µm
Mobile Phase	A: Pre-mixed (methanol: glacial acetic acid: water, 28:3:69)
Flow Rate	2.0 mL/min
Temp	45 °C
Wavelength	275 nm
Injection Volume	5 µL
Analyte	Acetaminophen, Aspirin and Caffeine

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.45 µm nylon filter, P/N 02542880.

A standard stock of USP acetaminophen (0.25 mg/mL), aspirin (0.25 mg/mL) and caffeine (0.065 mg/mL) was prepared using a solution of methanol and glacial acetic acid (95:5) as diluent.

An internal standard solution of benzoic acid in methanol (6 mg/mL) was prepared.

A 10 mL standard solution was prepared with 4 mL of standard stock solution and 0.6 mL of internal standard solution before being made up to the mark with diluent.

Results and Discussion

Acetaminophen, aspirin and caffeine have been successfully analyzed with an internal standard (benzoic acid) in under six minutes using a C18 (100 x 4.6 mm, 5 µm) column. The USP monograph specifies that a column with L1 packing (100 x 4.6 mm, 5 µm) be used.

L1 packing is defined as octadecyl silane chemically bonded to porous or non-porous silica. The C18 column used complies with the USP monograph and is suited to the analysis of acetaminophen, aspirin and caffeine as demonstrated by the results, Figure 2.

The injection volume was adjusted to 5 µL, the USP <621> allowed changes states injection volume can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits.[5]

The USP monograph system suitability requires that the relative standard deviation (RSD) of peak area and retention time for five successive replicates be no more than 2.0%, the tailing factor of the analyte peaks be no more than 1.2 and the resolution of all peaks be greater than 1.5. The data collected from the LC 300 HPLC system met all of these conditions (Table 2) and gave a repeatable separation for all four components.

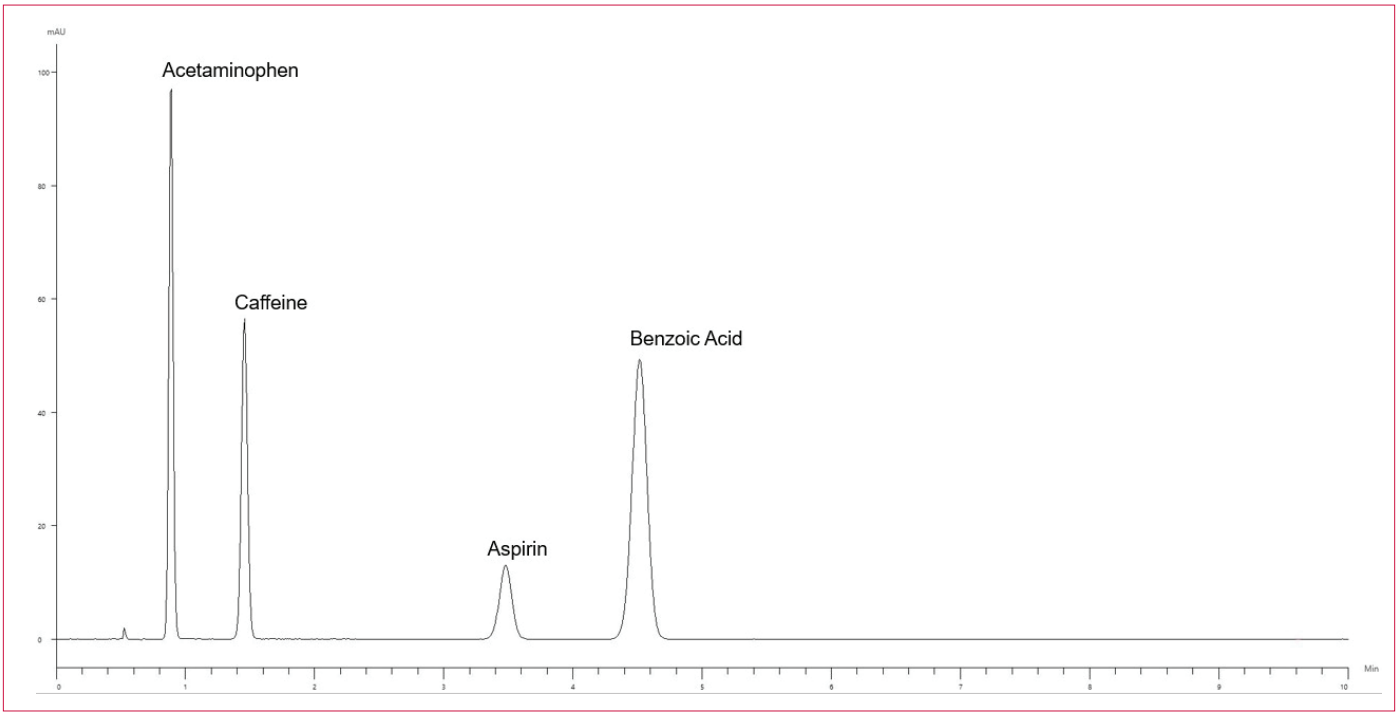

Figure 2. Alysis of acetaminophen, aspirin, caffeine with benzoic acid internal standard.

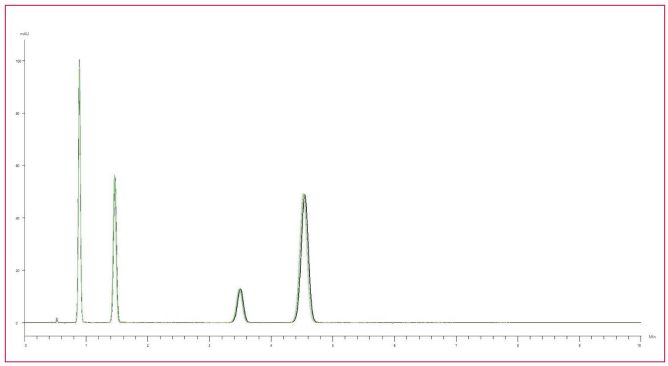

Figure 3. Overlay of five successive reps of Acetaminophen, Aspirin and Caffeine.

Table 2. Results summary. RSD calculated from five successive injections.

Suitability Requirement	Peak Area RSD (%)		Retention Time RSD (%)		Tailing Factor		Resolution	
Analyte	LC 300	USP	LC 300	USP	LC 300	USP	LC 300	USP
Acetaminophen	0.60	≤ 2.0	0.28	≤ 2.0	1.03	≤ 1.2	N/A	> 1.4
Aspirin	0.46		0.29		1.03		7.4	
Caffeine	0.34		0.34		0.99		14.5	
Benzoic Acid (Internal Standard)	0.49		0.27		0.99	N/A	5.0	

Conclusion

- Fast analysis of acetaminophen, aspirin and caffeine was carried out on a C18 (100 x 4.6 mm, 5 µm) column in under 6 minutes.
- The LC 300 system facilitates repeatable separations for acetaminophen, aspirin and caffeine meeting all suitability requirements in the USP monograph.
- Excellent peak shape and low %RSD for peak shape was obtained for all analytes and met USP requirements using the PerkinElmer LC 300 system.

Consumables

Component	Description	Part Number
HPLC Vials	2 mL Amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (Polypropylene) Cap with PTFE/Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable (100/pack)	02542890
Syringe Filters	0.45 µm Nylon Filter, 17 mm Diameter	02542880
PEEK Fittings	Finger-tight for 1/16" OD PEEK Tubing	09920513
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	N9306301

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